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(57) Abstract

The present invention relates, in general, to a novel therapeutic approach that involves the use of soluble, metabolically stable and pharmaceutically acceptable constructs to induce the immune system to produce immunoglobulins that can function as alternative receptors for a specific ligand. Since the immunoglobulins produced compete with naturally occurring receptors for the ligand, the availability of the ligand to its natural receptor can be regulated. Regulation of ligand availability makes possible modulation of the response produced upon binding of the ligand to its natural receptor.

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ALTERNATIVE RECEPTOR THERAPY

FIELD OF THE INVENTION

The present invention relates, in general, to a novel therapeutic approach that involves the use of soluble, metabolically stable and pharmaceutically acceptable constructs to induce the immune system to produce immunoglobulins that can function as alternative receptors for a specific ligand. Since the immunoglobulins produced compete with naturally occurring receptors for the ligand, the availability of the ligand to its natural receptor can be regulated. Regulation of ligand availability makes possible modulation of the response produced upon binding of the ligand to its natural receptor.

BACKGROUND

The immune system can be subdivided into two primary branches based upon the response under consideration and the type of cell mediating this response. Classically these responses are referred to as humoral (antibody or immunoglobulin mediated) and cellular responses. Lymphocytes that synthesize immunoglobulins are referred to as B-cells while lymphocytes that are responsible for modulating B-cell function and for direct cytotoxicity are referred to as T-cells.

The therapeutic approach of the present invention relates, at least in large part, to B-cell responses, i.e. antibody (Ab) production. Antibodies have two primary functions: 1) antigen (Ag) recognition, and 2) effector function. These functions correspond to two different portions of

the immunoglobulin molecule. The Fab portion of the molecule is responsible for recognition (Ag binding) whereas the Fc portion is responsible for effector functions. The type of Fc defines the class of immunoglobulin and the effector response produced when an Ab binds to an Ag. In general, effector functions require that the Fc region of the Ab be recognized and bound to a class-specific receptor by some other cell (macrophages, mast cells, basophils, polymorphonuclear leukocytes, etc.). This recognition most often requires the formation of immune complexes consisting of multivalent antigens bound by multiple Ab molecules. Single antigen-antibody pairs do not form immune complexes and therefore do not elicit effector responses. This is an important issue with respect to the present therapeutic approach since responses such as mast cell stimulation or immune complex production may be undesirable.

For immune complexes to form, at least two Ab recognition sites (epitopes) need to exist on a single antigen and be simultaneously available to two different immunoglobulin molecules. Small molecules (such as cocaine and other ligands to which the invention relates) cannot be bound simultaneously by two different Abs. As a result, immune complex formation or receptor crosslinking on cells cannot be achieved. Allergic responses to small molecular weight ligands such as penicillin, sulfa, procainamide, etc. can be attributed to the propensity of these compounds to covalently bind to proteins, thereby producing multiple epitopes for simultaneous Ab binding on a single macromolecule. Where the pharmacology, metabolism and chemistry do not allow for such interactions, multiple binding

sites on the same macromolecule are highly unlikely to occur. Without multiply substituted macromolecules, Ag/Ab complexes cannot be formed. As a result, risks such as anaphylaxis or severe allergic reactions to an administered small molecular weight ligand which could result from the induction of an immune response to the ligand by the production of antibodies are minimal.

There are five classes of immunoglobulins: IgM, IgG, IgE, IgA and IgD. Of these five classes, all but IgM (and a small amount of IgG of the IgG3 subclass) require the participation of T-cells or T-cell derived factors (cytokines) for their production. As a result, IgM production can be considered the sine qua non of a T-independent antibody response while the others can be classified as T-dependent antibody responses. Each of these immunoglobulin classes are different in their physiologic and kinetic characteristics. For example, allergic symptoms ranging from simple rhinitis to anaphylaxis can be attributed to IgE antibodies while immunoglobulins found in mucosal secretions are of the IgA class. Other classes have different attributes.

Upon first encounter with a novel antigen, naive B-cells expressing membrane bound receptors (albeit of low affinity) which recognize this antigen, begin to differentiate into plasma cells and secrete IgM. These IgM antibodies have a binding site which is identical to the binding site of the original B-cell receptor and have binding constants on the order of 10^{-5} to 10^{-6} molar. Circulating IgM titers produced in response to a single pr sentation of antigen peak at approximately five to seven days and fall slowly over the course

of several weeks (primary response). Repeated challenges with such an antigen (one that does not involve T-cells or T-cell dependent antibodies) do not invoke "booster" responses or memory phenomenon but interact with the immune system as if they were simple primary challenges eliciting primary responses. This is what is seen in response to simple antigens such as bacterial polysaccharides.

In order for the immune response to switch classes from IgM to IgG or IgE or develop higher affinity antibodies (class or isotype switching and affinity maturation), T-cells and T-cell factors must be involved. Once T-cells are involved, the type of antibody secreted changes initially from IgM to IgG, IgA or IgE (depending on the setting). IgG and IgA antibodies have binding constants ranging from 10^{-6} to 10^{-9} M while IgE affinities can be as high as 10^{-11} M. In addition, the kinetics of these responses are significantly different. IgG, for example, rarely is seen sooner than 10-14 days after initial challenge. However, significant titers may be present for weeks to months (or even years in some cases) and booster responses upon secondary challenge are the norm.

One skilled in the art will appreciate from the foregoing that immunoglobulins (or alternative receptors) with binding constants ranging from 10^{-5} to 10^{-9}, which are key to various embodiments of the present therapeutic approach, can be readily achieved. The persistence of these immunoglobulins in circulation allows for the intermittent dosing of the ligand-specific immunostimulatory constructs of the invention. The artisan will also appreciate that circulating immunoglobulins are ideally situated to interrupt or

block the delivery of active ligand to the target organ, for example, the brain or cardiovascular tissues. The effect of this interruption of delivery is amplified in the case of ligands (such as cocaine) that are subject to inactivation by, for example, circulating esterases.

Immunoglobulins (or purified Fab fragments) have been used successfully as alternative receptors for a number of toxicologic emergencies. As far as therapies currently approved for human use are concerned, all of these cases involve the use of exogenously administered antibody (see Smith et al, *N. Engl J Med* 294:797 (1976); Smith et al, *N Engl J. Med*, 307:1357 (1982); Spiegal et al, *Am Heart J* 109:1397 (1985); Wenger, et al, *J Am Coll Cardiol*, 5:118A (1985); Bonese et al, *Nature*, 252:708 (1974)). In contrast, the present invention involves the use of immunostimulatory constructs to induce the endogenous production of such antibodies. The advantages of this approach will be clear from the description of the invention that follows.

SUMMARY OF THE INVENTION

It is a general object of the invention to provide a novel therapeutic approach that involves regulating the availability of a specific ligand to its naturally occurring receptor, and thereby modulating the response produced upon binding of that ligand to its natural receptor.

It is a specific object of the invention to provide a method of treating drug abuse, for example, cocaine, opiate or nicotine abuse.

It is another object of the invention to provide a method of regulating the circulating level of endogenous hormones and growth factors. In this regard, specific objects of the invention include providing a method of regulating the levels of tumor-associated growth factors and thereby inhibiting tumor growth and, providing a method of regulating the levels of pituitary releasing hormones and thereby controlling fertility.

The method of the invention has broad applicability as will be appreciated by one skilled in the art from a reading of this disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1: Administration to mice of fluorescein (Fl) conjugated to dextran of 500,000 MW ($Fl\text{-}dex_{500}$) Δ---Δ IgM boost, ▲---▲ IgM control.

FIGURE 2: Administration to mice of $Fl\text{-}dex_{500}$; O---O IgG boost, O---O IgG control.

FIGURE 3: Injection of mice with constructs, IgM Ab titer (FIG. 3a), IgG Ab titer (FIG. 3b); $Fl\text{-}dex_{500}$ boost: O---O; $Fl\text{-}dex_{500}$ saline: O--- O; (Fl + peptide)-dex_{500} boost: ▲--- ▲; (Fl + peptide)-dex_{500} saline: Δ--- Δ.

FIGURE 4: Structure of cocaine.

FIGURE 5: Cocaine analogs.

FIGURE 6: Opiate derivatives.

FIGURE 7: Opiate modifications needed for linking to immunoconjugates.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of inducing the immune system to produce immunoglobulins in high enough titer and with the appropriate affinity distribution to serve as alternative receptors for a particular ligand and to thereby control the availability of that ligand to its natural receptor. The invention further relates to constructs suitable for use in such a method. The chemistry of the constructs of the present invention can be readily altered with the result that the affinity and class distribution of the resulting antibodies can be controlled.

The method of the invention is described herein in some detail with reference to a treatment for drug abuse, specifically, cocaine and morphine (opiate) abuse. The method has wide applicability, however, in that it can be employed to control the availability of endogenous ligands, in addition to compounds exogenously administered for therapeutic and non-therapeutic purposes. In the context of endogenous ligands, the method can be used to control the availability of, for example, hormones and growth factors, and thereby to control, for example, fertility (by controlling availability of pituitary releasing hormones) and tumor growth (by controlling availability of tumor growth factors). Potentially, the method can be used to regulate the availability of any of a number of small molecules and thereby control the effects elicited upon

binding of such molecules to corresponding naturally occurring receptors.

An example of the above-described approach is the use of peptide-protein immunoconjugates as a vaccine to raise antibody titers to the decapeptide: Gln-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly which appears to regulate gamete formation and activation in cattle and other animals. Antibodies directed to this peptide will depress fertility in the immunized animals as long as circulating antibodies are present (Hoskinson et al, *Australian J. Biotechnology* 4(3):166 (1990)). Antibodies directed to this peptide or other peptides such as follicle stimulating hormone (FSH) and/or luteinizing hormone (LH) can be expected to have the same type of an effect in humans. Similarly, the existence of antibodies to human chorionic gonadotropin (HCG) can also be expected to suppress fertility by preventing the normal hormonal changes that accompany fertilization, implantation and gestation. Alternatively, hormone and/or growth factor sensitive malignancies such as estrogen sensitive breast cancers, testosterone sensitive prostate cancers, melanocyte stimulating hormone (MSH) responsive melanomas, etc. are anticipated to respond to antibodies directed against these "growth factors" or to antibodies directed against modulators of the release of such factors.

Immunostimulatory constructs suitable for use in the present method can be designed based on technology developed by the Dintzis' which is described in detail in Application No. 07/808,787 (see also Dintzis et al, *Proc. Natl. Acad. Sci. USA* 73:3671 (1976); Dintzis et al, *Proc. Natl. Acad.*

Sci. USA 79:884 (1982); Dintzis et al, *J. Immunol.* 131:2196 (1983); Vogelstein et al, *Proc. Natl. Acad. Sci. USA* 79:395 (1982)). That technology defines the molecular and physical requirements for a given macromolecular construct to be stimulatory versus non-stimulatory as far as T-independent antibody production is concerned. In general, the following parameters must be met in order for a given construct to be stimulatory:

i) the overall molecular weight of the macromolecular construct consisting of a biologically inert backbone (carrier or scaffold) and the relevant antigenic epitope in question must be greater than 100,000 daltons, optimal responses are seen around 300,000-500,000 daltons; and

ii) the total number of antigenic epitopes per macromolecule must be greater than 20, with the more the better.

Examples III and IV, below, include detailed descriptions of the design of constructs suitable for use in the present method. While Example III makes specific reference to cocaine and Example IV to opiate, one skilled in the art will appreciate that the same approach can be applied to other ligands, including endogenous hormones and growth factors. Example III further includes details of treatment regimens, etc., in the context of a method for treating cocaine abuse. The artisan will understand, however, that the same principles apply to the other treatment protocols encompassed within the scope of the invention.

As noted above, the present invention is described in some detail with reference to a treatment of cocaine abuse. Other forms of drug

abuse that can be expected to be responsive to treatment using the present method, however, include opiate, benzodiazepine, phencyclidine and nicotine dependence.

As to cocaine abuse, the present method can be used to induce the production of circulating antibodies specific for this ligand that compete effectively with tissue-bound receptors for cocaine and in so doing, block its physiologic and psychologic effects by controlling its availability to the central nervous system (CNS). One skilled in the art will appreciate that ideally an alternative receptor therapeutic approach to cocaine, nicotine or opiate abuse should have the characteristics described below, which characteristics are fulfilled by the present method and constructs as indicated:

1) The induction of Ab should be easy, safe, effective and without significant side effects, either local or systemic. Commonly used adjuvants are not acceptable in normal clinical practice because of their high incidence of local inflammatory reactions and because repeated administration carries significant risks for both local as well as systemic reactions.

Immunizations using the constructs described herein are particularly well-suited for the induction of T-independent Abs and do not require the use of adjuvants of any kind. Local or systemic reactions to these agents have never been observed.

2) The Ab population should be of moderate to low affinity. If high affinity Abs are produced, their ability to be saturated and, as a result, neutralized as competitive receptors would obviate their utility. Advantageously, these

alternative receptors have rapid (1-5 millisecond) on and relatively slow (100-500 second) off rates (nominal affinity constants on the order of 10^{-5} to 10^{-6} M). This allows for rapid binding and slow (but measurable) release, thereby allowing for receptor regeneration. For this therapy to be effective, the alternative receptor need not bind all of the available drug for long periods of time; all it need accomplish is to retard the availability of cocaine (nicotine or opiate) to the CNS by blunting peak free levels. Since, as noted above, cocaine is rapidly inactivated by a number of circulating esterases, the primary utility of these alternative receptors is to lower the acute free serum levels of the drug that are normally seen with routine cocaine use and which are associated with both the euphoric and subsequent dysphoric psychopharmacologic effects.

IgM and low affinity IgG Abs (IgG3), produced in accordance with the present invention, have binding constants on the order of 10^{-5} to 10^{-6} M with fast on and slow (but measurable) off characteristics and IgG3 passes placental barriers without difficulty thereby potentially affording significant fetal protection.

3) Circulating Ab titers should be of the same order of magnitude (on a molar ligand binding basis) as the peak free drug level seen in serum under normal use. This does not mean that the concentration of Ab should be the same as the concentration of drug at steady state, but that, preferably, the total amount of ligand binding equivalents of immunoglobulin in the circulation should be approximately equal to the dose of drug administered on a molar basis.

IgM, produced in accordance with the present invention, has a ligand binding capacity of 10 binding sites per protein molecule and levels produced (taking this binding capacity into account) can be expected to be adequate for purposes of the present invention.

4) Ab titers should be relatively long lived requiring only intermittent enhancement or induction. Repeated stimulation should not change the pharmacologic characteristics of the competitive receptor, nor should repeated induction or stimulation carry with it any significant risk of toxicity.

IgM levels, produced by the present method, have adequate serum half lives but, more importantly, repeated stimulation or induction using the constructs described herein does not produce affinity maturation or class switching (both potential detriments in the present method). Changes in affinity or class could have serious deleterious consequences including changes in receptor affinity such that the Abs can be inactivated by ligand binding and the production of undesired immunologic side effects.

5) Administration of drug in the presence of Ab should not have any untoward effects.

IgM (because of its relatively low affinity) does not normally produce Ag-Ab complexes (unless epitope expression on the antigen is highly multivalent) or untoward immune-mediated side effects. In the present method, the immune response can be controlled so that IgM is the primary immunoglobulin produced. Accordingly, side effects in th presence of drug can be expected to be minimal.

One skilled in the art will appreciate that unless there is rapid access to the CNS, the behavioral effects of cocaine (and likewise nicotine and the opiates) are blunted. By interfering with this access, either by decreasing free serum levels, by altering the "volume of distribution" of the drug or by enhancing metabolic or elimination kinetics relative to blood brain barrier penetration, the behavioral consequences of drug use can be altered. All of these aspects of substance abuse pharmacology are expected to be amenable to modification by the induction of alternative immunoglobulin receptors using the present invention.

The non-limiting Examples that follow describe certain aspects of the invention in further detail.

Example I
IgM and IgG Production with Simple Conjugates

As discussed above, simple constructs (conjugates) consisting of a biologically inert backbone (high molecular weight dextran >100,000 daltons has been used as a plasma expander) and a cocaine analog (see Example III) can be used for epitope-specific T-independent Ab induction. These conjugates can be expected to produce IgM and IgG3 Ab responses analogous to those seen for other simple haptens. The data that follow relate to such constructs.

When Balb/c mice were injected with the small hapten, fluorescein (Fl), conjugated to dextran of 500,000 MW (referred to as Fl-dex_{500}) at a high substitution ratio, IgM but not IgG Ab

responses to Fl developed within 7 days and persisted for at least 6 weeks (FIGS. 1 and 2).

In the situation where a second dose of Fl-dex_{500} was administered to these mice, the IgG response was still unaffected whereas the IgM response was boosted but did not exceed the magnitude of the primary response (FIGS. 1 and 2). Therefore, it appears that this Ag does not cause the induction of memory B cells nor affinity maturation of the Ab response which occurs as a consequence of memory development.

This observation indicates that this type of immunostimulatory construct (small hapten on a dextran backbone) is ideally suited for use in the alternative receptor therapy of the invention since it raises predominately IgM Abs of low affinity and higher affinity Abs do not develop over time when multiple injections of Ag are given. The fact that there did not appear to be a memory response is noteworthy, and, if these antibodies are consistently produced and available, transplacental passive immunization can be expected. Where Ab titers achieved with these simple haptens are insufficient to adequately blunt the free serum levels of the ligand, alternative approaches can be employed.

Example II

IgM and IgG Ab Production with Complex Conjugates

To determine whether the presence of a synthetic peptide would influence the immune response to Fl, Fl and molecules of the synthetic peptide have been co-conjugated to the same dextran backbone. The peptide chosen has been one produced

by trypsin digestion of chicken ovalbumin, encompassing residues 323-339, which is well-documented in the literature as a potent T cell epitope. It was questioned whether the presence of this epitope on the same backbone as Fl would induce a predominantly IgG primary response against Fl, as well as memory induction and affinity maturation, because peptide-specific T helper cells could co-operate with Fl-specific B cells to induce the class switch from IgM to IgG as well as the other association phenomena. It did.

Mice injected with these complex constructs [(Fl + peptide)-dex_{500}] had higher IgG anti-Fl responses than those which received only Fl-dex_{500} conjugates (FIG. 3a and b). IgM and IgG Abs were also raised against the peptide itself (data not shown).

On secondary injection, it appeared that the (Fl + peptide)-dex_{500} conjugate boosted the anti-Fl IgG Ab response exceeding both the primary response and the secondary response to Fl-dex_{500} (FIG. 3a and b). This suggests that this alternative strategy could be used if necessary to increase the anti-hapten response.

One problem that could arise by using a foreign peptide in a complex construct with a small hapten is that the deleterious effects of the Ab response generated against the peptide itself could result, especially if this peptide were a sequence from a commonly encountered Ag such as ovalbumin. To avoid the risk of anaphylactoid-type episodes, peptides based on T-cell epitopes from vaccines, for example, against tetanus, diptheria or pertussus, or commonly experienced viral pathogens (measles, mumps, rubella, chicken pox, influenza A or B, etc)

can be employed. While the foregoing comments include specific reference to fluorescein, one skilled in the art will appreciate that cocaine, morphine or nicotine analogs, for example, will behave as B-cell epitopes analogous to fluorescein.

Example III
Induction of Alternative Receptors to Cocaine

A. Development of soluble, metabolically stable and pharmaceutically acceptable agents for alternative receptor induction.

Ab stimulation is critically dependent on the biologic stability of the construct used to stimulate the immune response. One of the advantages of adjuvants is that they decrease the metabolic degradation of the immunogen and thereby prolong its biological activity. However, many of the problems with adjuvants such as local inflammatory reactions, tissue injury and discomfort are at least in part due to their "depot" like behavior. The macromolecular constructs used in the method of the present invention are designed to be freely soluble and easily distributed by intravenous, subcutaneous or intramuscular injection so as to eliminate local side-reactions or inflammatory stimuli. This is particularly important for agents that are to be used on a regular or repeated basis. In addition, Ag/Ab complexes or "serum sickness" type reactions are unlikely to occur since low to moderate affinity antibodies will be produced.

Cocaine is rapidly degraded by circulating esterases which would alter the chemical nature of

the epitope in question. As a result, metabolically stable cocaine analogs can be used that bear sufficient homology to cocaine to induce production of Abs that recognize the intact cocaine molecule. Given the fact that low to moderate affinity antibodies are desired, use of molecular analogs is advantageous since chemical moieties such as ketones, amides or phosphates, bear sufficient homology to esters to induce Abs that recognize esters for catalytic and other purposes and are relatively metabolically inert.

One skilled in the art will appreciate that selection of preferred constructs is best made using outbred strains of mice since such mice decrease the potential complications of the unique genetics of inbred mouse strains, more accurately reflecting an "outbred" human population.

The intact cocaine molecule can be subdivided into four regions, as shown in FIG. 4. The immunoglobulins (alternative receptors) generated by the present method must be capable of recognizing and binding the active compound at least as well as (if not better than) the inactive metabolites. For example, regions 2 and 3 are highly sensitive to metabolic degradation by circulating serum esterases. As a result, conjugates utilizing compounds (haptens) that retain esters in these positions will be rapidly degraded, thereby preferentially generating Abs to cocaine metabolites as opposed to the active compound. Clearly, linking the hapten to a backbone through an ester linkage would be undesirable. Finally, the stereochemistry of positions (a) and (b) may or may not be important for the ultimate immunogen even

though they are known to be critically important for the biologic of cocaine (see below).

1. Conjugate design, synthesis and analysis

Several different strategies can be employed to take advantage of the structure of cocaine. In all of the compounds shown in FIG. 5, a free carboxyl group is used to link the hapten to the polymeric carrier. In general, regions of the molecule that are closest to the backbone carrier are relatively "invisible" to immune recognition and do not play a major role in defining specificity or affinity. As a result, compounds that use modifications of regions 2 or 3 that are metabolically stable (compounds 1 and 2) for conjugation generate Abs that recognize the other regions of the molecule. This has certain disadvantages in that both the active compound as well as its metabolites retain this structure, therefore, the Abs generated are unlikely to be able to discriminate between these alternative ligands. On the other hand, the stereochemistry of the molecule at carbon (a) for compounds linked through region 2 or, alternatively, at carbon (b) for compounds linked through region 3, become less important as these carbons are unlikely to be "seen" by the immunoglobulin receptor.

Another approach is to modify positions 1 or 4 for conjugation to a carrier. In this case, Abs would be more likely to be generated that could discriminate between active and inactive compounds, however the stereochemistry at carbons (a) and (b) would then begin to play a role. Obviously,

compounds utilizing modifications in regions 1 or 4 for linking need to be yet further modified in regions 2 and 3 in order limit enzymatic breakdown. Candidate compounds for this type of chemistry are compounds 3-7.

Finally, compounds that change the ester at region 3 to a phosphonate (phosphonates are transition state analogs for compounds that undergo esterase cleavage, compounds 8-13) are advantageous in that they generate Ab's that are not only specific for active compounds but also potentially have "catalytic" qualities. To the extent that they retain this type of "catalytic binding", they satisfy the criteria of active compound specificity in addition to fast off rates (in this case with ester hydrolysis and the release of an inactive molecule). In reality, actual catalysis is less important than the specificity for active compound that would be potentially available from these types of Abs. It is important to realize, however, that in these cases the stereochemistry at the appropriate carbon will be critical for the activity of the resulting immunoglobulins.

Target haptens, are conjugated to carrier molecules of various known immunogenic sizes (greater than 150,000 daltons). Dextran, Ficoll and polyacrylamide backbones of comparable molecular weights can be used to assess the contribution of backbone to the behavior of the constructs. These carriers can be modified to have the appropriate chemistry for conjugation. It should be noted that the haptens synthesized should also be tested for pharmacologic activity as cocaine agonists or antagonists themselves.

2. **Ab production, characterization and pharmacology**

Conjugates can be injected into mice using immunization protocols described in Application No. 07/808,797 in order to characterize their immunogenicity. Ab titer and affinity can be assessed to both the ligand used to raise the Ab response as well as to cocaine itself (competition and direct ligand binding). In addition, the distribution of immunoglobulin class (IgM, IgG1, IgG2, etc) over time can be assessed. For those conjugates that might be expected to generate at least a small proportion of catalytic Abs, sera can be tested for actual esterase activity in addition to Ag binding capabilities. Finally, the effect of repeated dosing of the conjugate and exposure to free ligand or cocaine on antibody titer, affinity and class for a selected group of conjugates that develop the higher titers and a range of affinities can be evaluated.

If complex conjugates are seen to be desirable (conjugates incorporating more complex structures designed to induce T-cell help), these can be easily synthesized using standard techniques.

Example IV

Induction of Alternative Receptors to Opiates

Synthesis of immunogenic constructs suitable for use in alternative receptor therapy to opiate dependence and abuse requires the modification of existing opiates for linking to an immunogenic backbone in order to generate antibodies capable of recognizing the general class of morphin

based drugs of abuse. The interaction of substances of abuse containing the morphine alkaloid ring system with opiod receptors is well defined. Although the structure activity relationships for drugs such as morphine and heroin are well known, they are largely irrelevant to the purpose of generating a specific hapten which can be used in appropriately sized and constitued immunogenic constructs. Illustrated in FIGURE 6 are examples of the types of modified morphine derivatives that can be used for this appliction.

One of the preferred types of chemistries that has been employed in the generation of immunogenic conjugates is the formation of maleimide containing backbone carriers which then can be reacted with thiol containing haptens. This type of reaction results in the generation of metabolically stable thioether linkages between the hapten and the carrier. The synthesis of modified morphine based haptens for the formation of these types of conjugates is illustrated in FIGURE 7a or b. In each case, the reactive thiol is protected by an easily removable sulfhydral protecting group (nitropyridinesulfenyl (Npys), in compound E, FIGURE 7a and benzyl (Bn) in compound B, FIGURE 7b). Once made, these compounds can be deprotected and reacted with the maleimide containing carriers described previously and used to induce the production of alternative receptors in a manner similar to that described above.

* * *

The entire contents of the references cited hereinabove are incorporated herein by reference.

One skilled in the art will appreciate from a reading of the foregoing disclosure that various changes in form and detail can be made without departing from the true scope of the invention.

WHAT IS CLAIMED IS:

1. A method of modulating a response produced in an animal upon binding of a ligand to a receptor comprising administering to said animal a conjugate that induces the immune system of said animal to produce antibodies to said ligand,

wherein said conjugate has a molecular weight of at least 100,000 daltons and comprises at least 20 antigenic epitopes of said ligand bound to a biologically inert backbone, and

wherein said conjugate is administered under conditions such that said antibodies are produced, which antibodies bind to said ligand and thereby reduce the availability of said ligand to said receptor.

2. The method according to claim 1 wherein said ligand is an exogenously administered drug.

3. The method according to claim 2 wherein said drug is an opiate, benzodiazepine, phencyclidine, cocaine, or nicotine.

4. The method according to claim 3 wherein said drug is cocaine.

5. The method according to claim 3 wherein said drug is an opiate.

6. The method according to claim 1 wherein said ligand is a hormone.

7. The method according to claim 6 wherein said hormone is a pituitary releasing hormone.

8. The method according to claim 6 wherein binding of said antibodies to said hormone reduces fertility.

9. The method according to claim 1 wherein said ligand is a growth factor.

10. The method according to claim 9 wherein said animal is a tumor-bearing animal and growth factor is a tumor growth factor.

11. The method according to claim 10 wherein said antibodies bind to said tumor growth factor and thereby inhibit growth of said tumor borne by said animal.

12. The method according to claim 1 wherein said conjugates further comprise T-cell epitopes.

13. The method according to claim 12 wherein said T-cell epitopes are derived from tetanus, diptheria or pertussus.

14. The method according to claim 12 wherein said T-cell epitopes are derived from a virus to which said animal has been exposed.

15. The method according to claim 14 wherein said virus is selected from the group

consisting of measles, mumps, rubella, chicken pox, influenza A and influenza B.

16. A conjugate having a molecular weight of at least 100,000 and comprising at least 20 antigenic epitopes of an opiate, benzodiazepine, phencyclidine, cocaine or nicotine bound to a biologically inert backbone.

17. A conjugate having a molecular weight of at least 100,000 and comprising at least 20 antigenic epitopes of a hormone bound to a biologically inert backbone.

18. A conjugate having a molecular weight of at least 100,000 and comprising at least 20 antigenic epitopes of a growth factor bound to a biologically inert backbone.

19. The conjugate according to one of claims 16-18 wherein said conjugate further comprises T-cell epitopes.

20. The conjugate according to claim 19 wherein said T-cell epitopes are derived from tetanus, diptheria or pertussus.

21. The conjugate according to claim 19 wherein said T-cell epitopes are derived from a virus to which said animal has been exposed.

22. The conjugate according to claim 21 wherein said virus is selected from the group consisting of measles, mumps, rubella, chicken pox, influenza A and influenza B.

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FIGURE 1

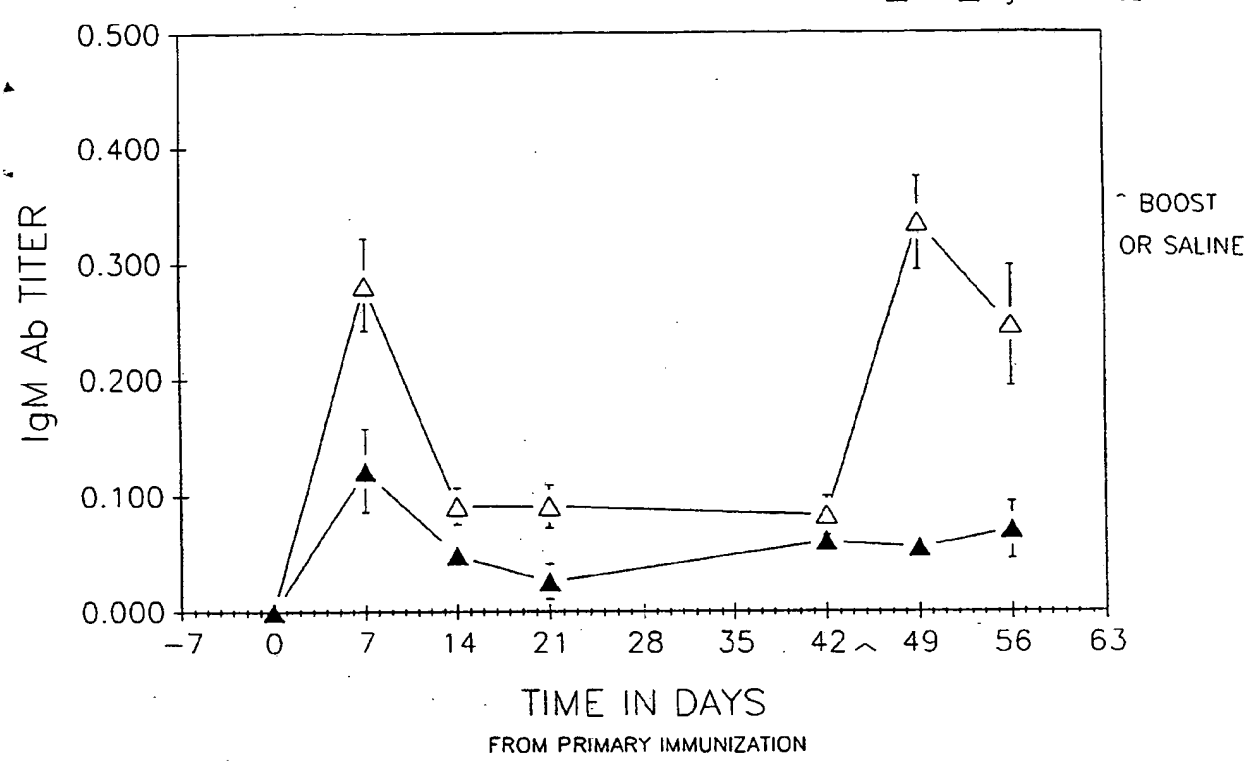

FIGURE 2

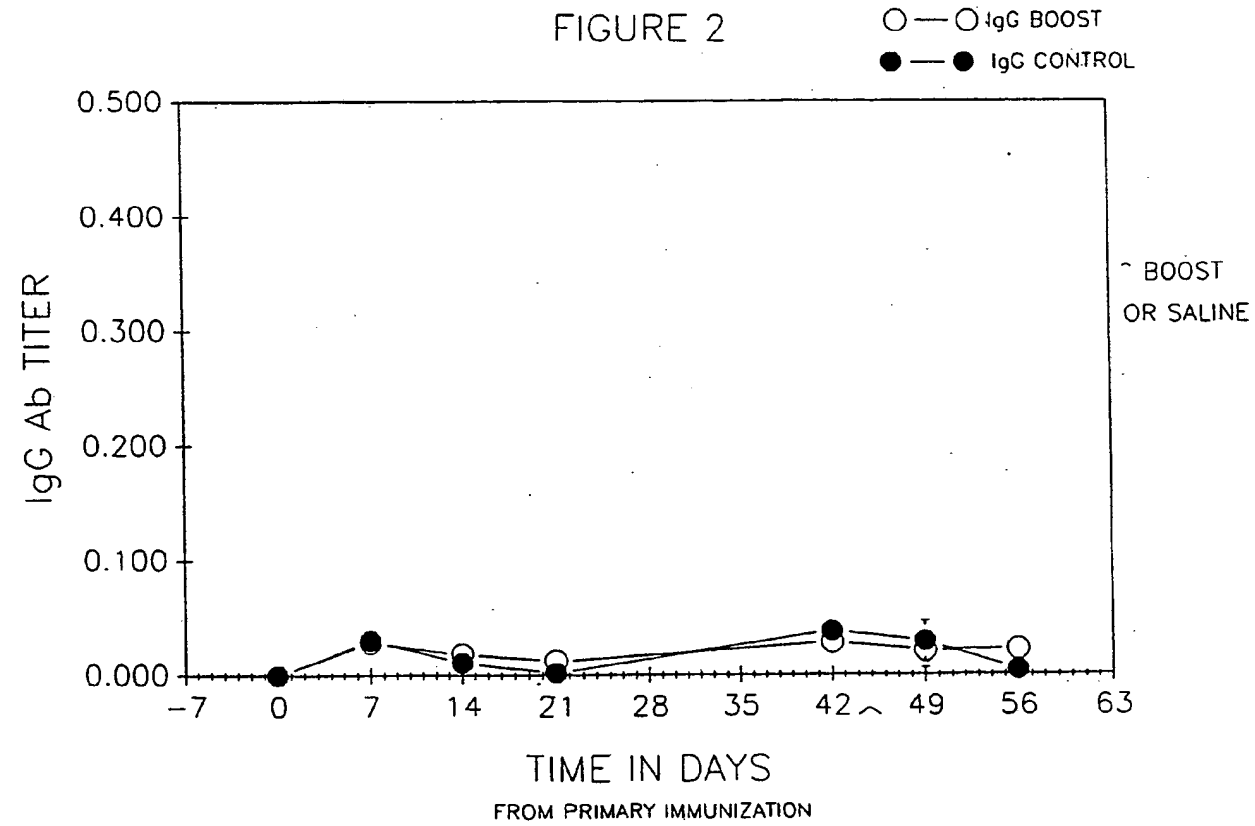

FIGURE 3a.

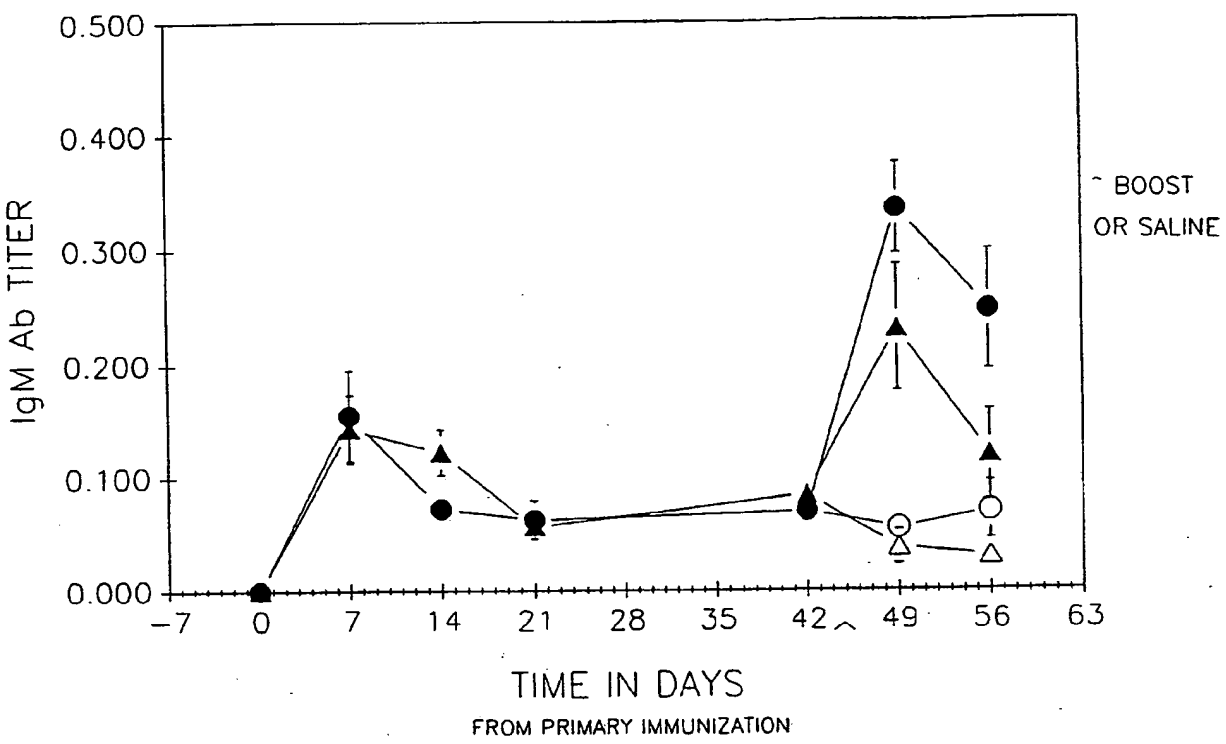

FIGURE 3b.

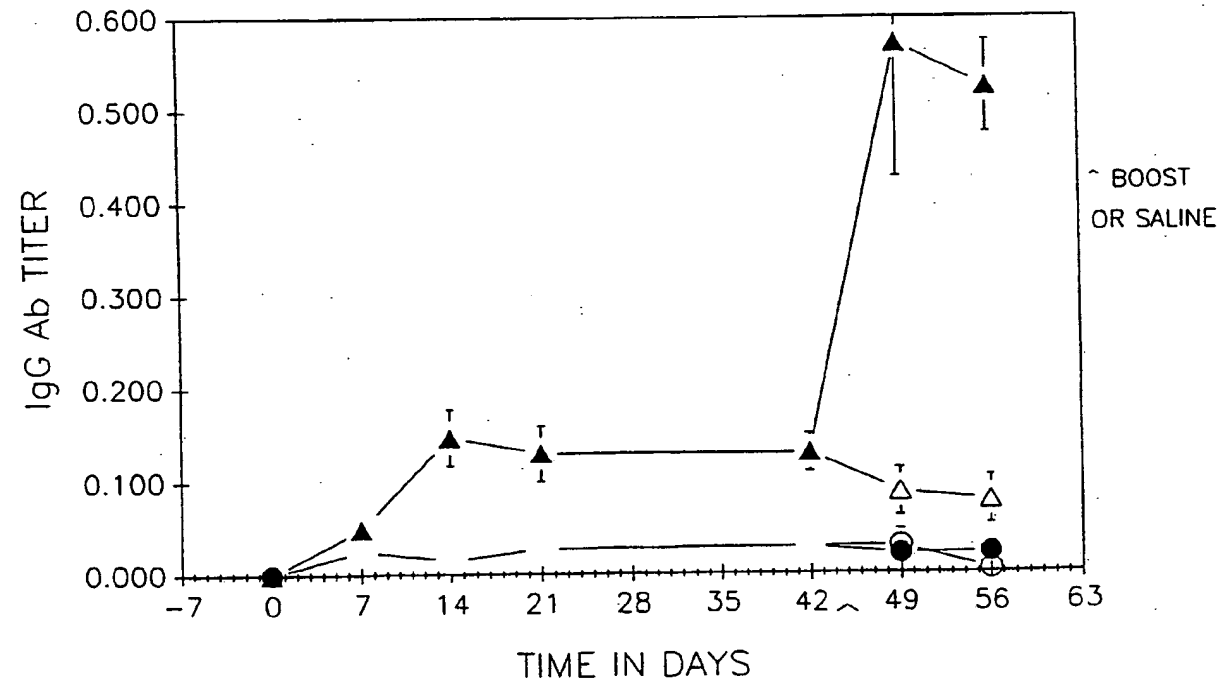

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FIG. 4

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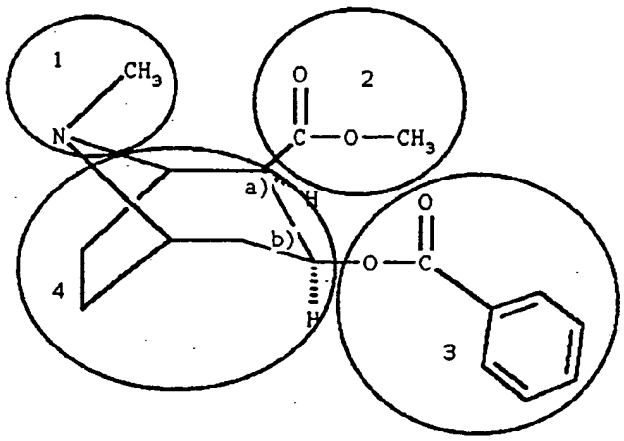
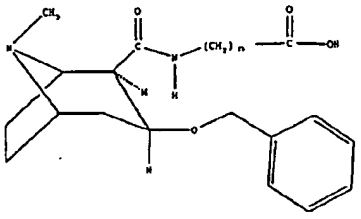


FIG. 5



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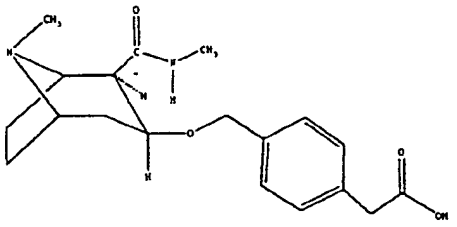

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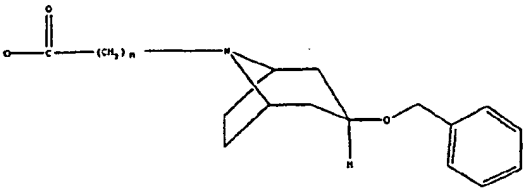

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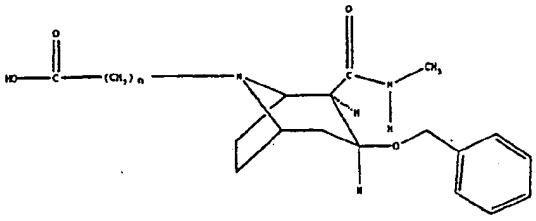

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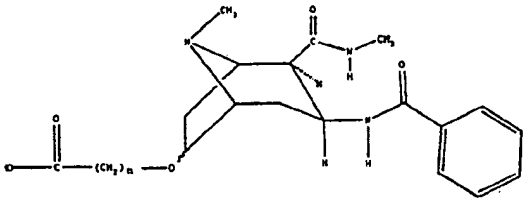

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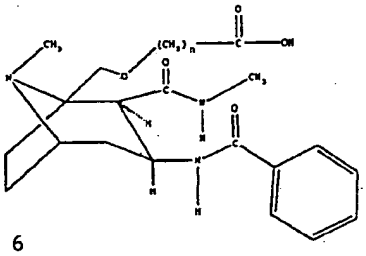

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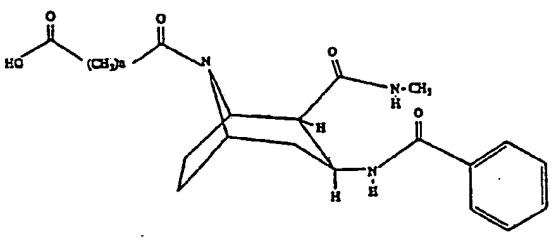

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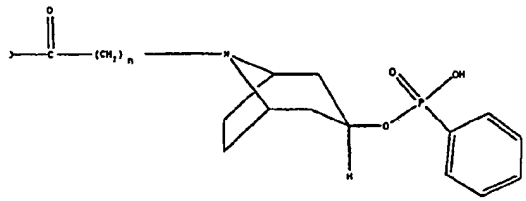

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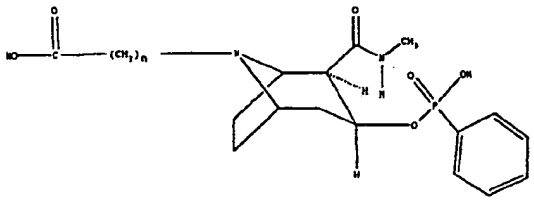

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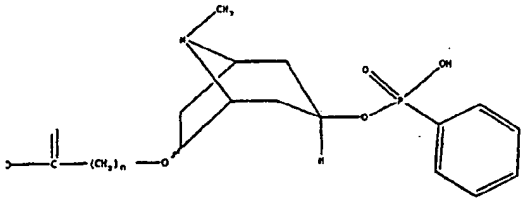

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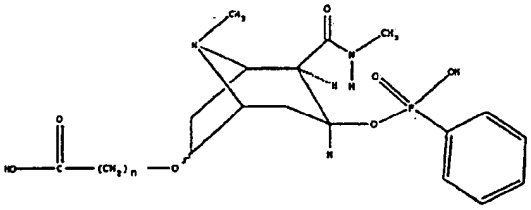

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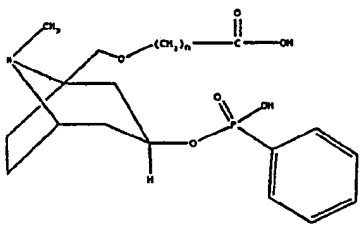

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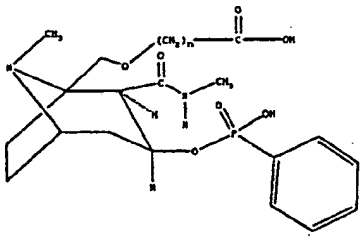

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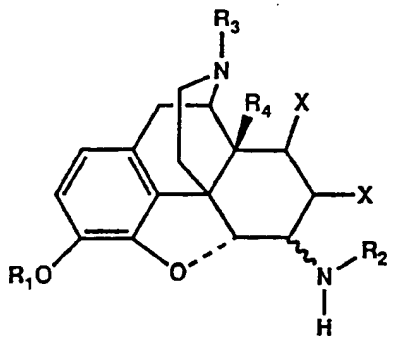


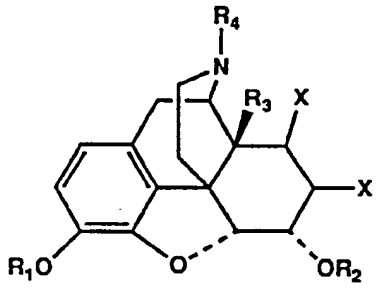
R_2= $-(CH_2)_n$-SH n=1 to 12

or

$-CO(CH2)_nHNCO(CH_2)_mSH$ n=1 to 12
m=1 to 12

or $-OC(CH_2)_nSH$ n=1 to 12.

R_1=H, CH_3, CH_3CO; R_4=H, OH;
R_3=CH_3 , X=H or H_2



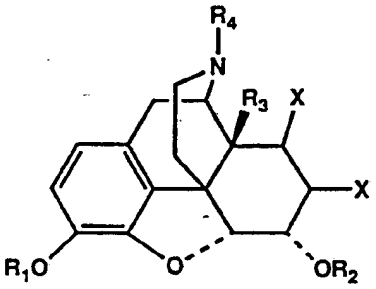
R_4= $-(CH_2)_n$-SH n=1 to 12

or

$CO(CH2)_nHNCO(CH_2)_mSH$ n=1 to 12
m=1 to 12

or $CO(CH_2)_nSH$ n=1 to 12.

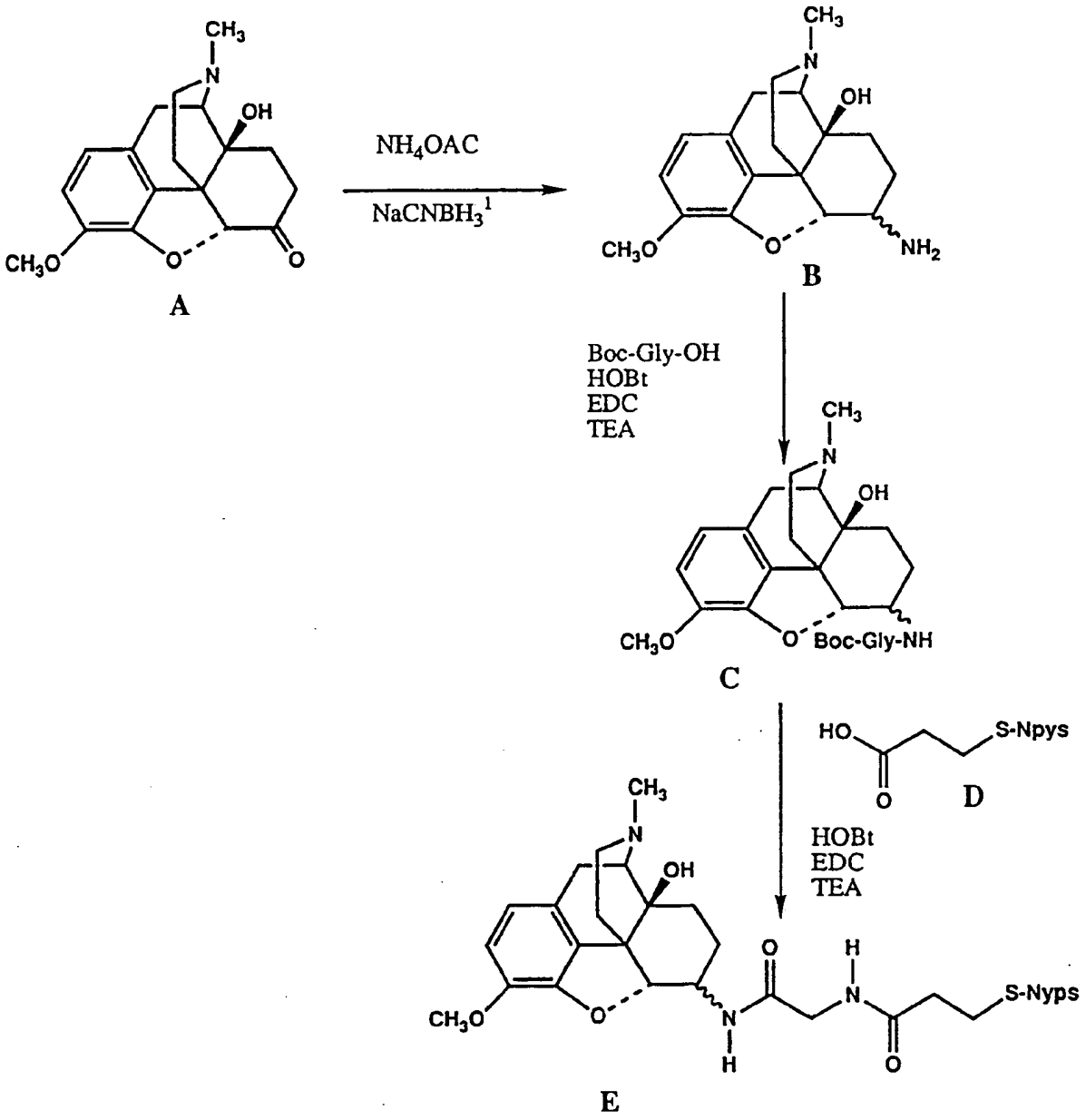
R_1=H, CH_3, CH_3CO; R_2=H,CH_3, CH_3CO R_3=H, OH; X=H or H_2



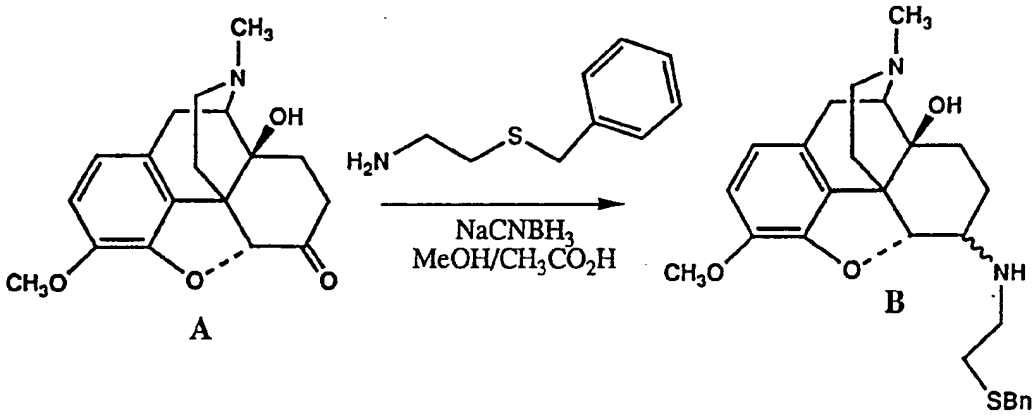
R_1= $-(CH_2)_n$-SH n=1 to 12
$-CH_2CO(CH_2)_mSH$ m=1 to 12

R_4= CH_3; R_2=H,CH_3, CH_3CO R_3=H, OH; X=H or H_2

Real examples have been synthesized as illustrated:



1. J. B. Jiang, R. N. Hanson, P. S. Portoghese J. Med. Chem. 20 1100-1102 (1977).


CH3
N
OH
CH3O
O
O
A
H2N
S
NaCNBH3
MeOH/CH3CO2H
CH3
N
OH
CH3O
O
NH
SBn
B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04644

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/00
US CL :424/88
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOGUE, SEARCH TERMS:IMMUNON, DINTZIS, EPITOPE DENSITY

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	THE FASEB JOURNAL, VOLUME 4, NUMBER 3, ISSUED 26 FEBRUARY 1990, BAGASRA ET AL, "A POTENTIAL VACCINE FOR COCAINE", PAGE A493, SEE ENTIRE ABSTRACT.	1-4,16 5-15,17-22
Y	THE JOURNAL OF IMMUNOLOGY, VOLUME 143, NUMBER 4, ISSUED 15 AUGUST 1989, DINTZIS ET AL, "THE IMMUNOGENICITY OF SOLUBLE HAPTENATED POLYMERS IS DETERMINED BY MOLECUILAR MASS AND HAPTEN VALENCE", PAGES 1239-1244, SEE ENTIRE DOCUMENT.	1-22
Y	HARLOW ET AL, "ANTIBODIES A LABORATORY MANUAL", PUBLISHED 1988 BY COLD SPRING HARBOR LABORATORY (N.Y.), PAGES 130-132, SEE ENTIRE DOCUMENT.	12-15, 19-22

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search
27 July 1993

Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04644

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP, A, 0,378,881 (ENIRICERCHE) 25 JULY 1990, SEE ENTIRE DOCUMENT.	12-15, 19-22
Y	US, A, 4,384,995 (STEVENS) 24 MAY 1983, SEE ENTIRE DOCUMENT.	6-13, 17-20